Single Cell Genomics Capabilities in the SOM Genome Analysis & Technology Core

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INTRODUCING

The Genome Analysis & Technology Core (GATC) Single Cell Library prep Sequencing Services

The Genome Analysis and Technology Core's continuous mission is to enable high throughput Next Generation Sequencing projects for both novice and experienced users alike. The core staff endeavor to provide timely assistance in all facets of experimentation from project design/execution to data analysis/reporting. Our goal is to facilitate institutional collaborations as well as provide staff/students training and educational opportunities.

This poster presents highlights of GATC's single cell sequencing services: These are uniquely based on 10X Genomics applications that enable researchers to profile a wide range of molecule alone or in combination, including DNA, RNA and protein. Single cell experiments are complex and require careful planning. Samples quality will impact the outcome, more so than for bulk RNA, and may require coordination between more than one core. However, it is a powerful tool that help researchers dissect complex biological systems.

LIST of GATC SERVICES

All project must be approved by the PI before the core can start processing the samples. Please contact us if you have any questions regarding project design, application support, service request, grant and publications support. Our service requests are available via the iLab portal.

Consultation & Training Services

Quality Control/Assurance Services
- qPCR-NGS library prep QC
- MiSeq-QC run 300 cycle Nano or Micro Kit
- NanoVue and Qubit
- Agilent - Tape Station - RNA/DNA integrity
- Agilent - Bioanalyzer - DNA HS

PCR Services
- qPCR - gene expression (TaqMan or Syber Green)
- qPCR-SNP genotyping
- ddPCR- quantitation and CNV analysis

Bulk RNA/DNA Library Prep services
- Metagenomic, small genome and 16S
- mRNAseq , total RNA seq w/ribo depletion
- small RNA
- Custom

GridION X5

Oxford Nanopore Long Reads

Single Cell/Nuclei 10X Genomics Library Prep services (see Vignette detail)

NGS Run Services (see Vignette detail)

CONTACT US

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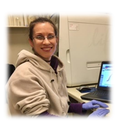


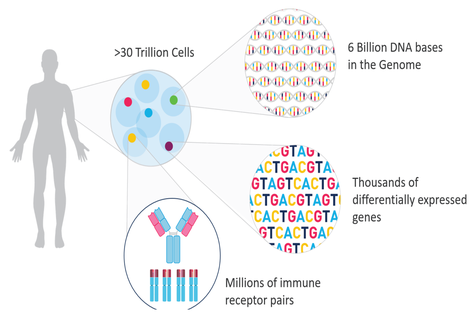
We are located in Pinn Hall, University of Virginia School of Medicine
1340 Jefferson Park Avenue. Charlottesville VA 22908

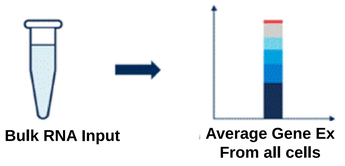
ACKNOWLEDGEMENTS
For publications containing data generated by the core:
Please include the core full name and core RRID number in the method section:
e.g. Genome Analysis and Technology Core, RRID:SCR_018883

10X Genomics Single Cell Analysis Services

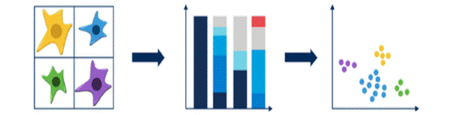
Single cell RNA-Seq opens a whole new field in biology by allowing the study of cell-to-cell transcriptome heterogeneity. It enables the discovery of cellular differences typically masked by standard, bulk RNA sequencing.

Genomic Complexity

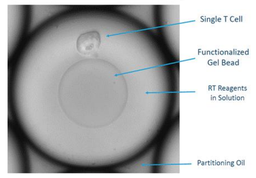




Sc. Gene Expression

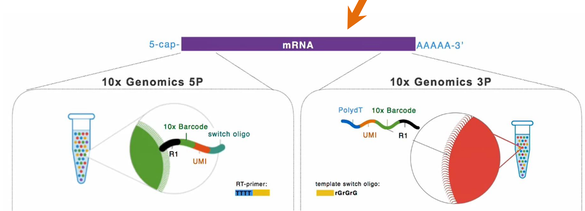


*Using the 10X Genomics Chromium Controller single cell GEM technology



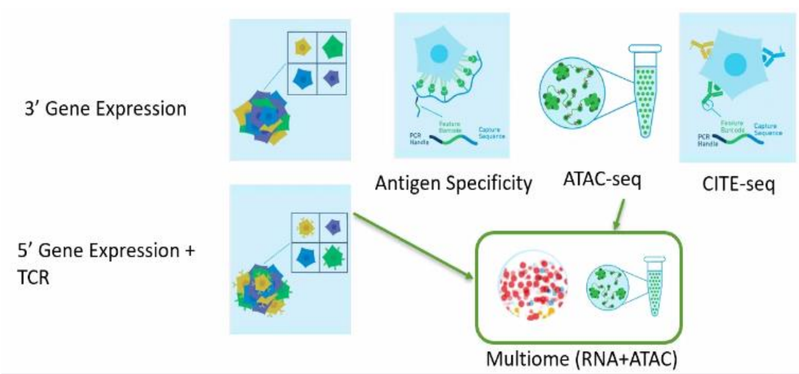
Partitioning &Cell Barcoding

The technology can capture from a single cell suspension prepared by the user, 500 to 10,000+ cells per well and generate 5' or 3' indexed gene expression libraries that can be run on any Illumina sequencing platforms.



Current 10x Genomics Assays

Assay	Analyte	Purpose
RNA-seq	RNA	Cell identification and phenotyping
ATAC-seq	Open chromatin regions	Cell identification and regulation
CITE-seq	Protein	Cell identification
Immune Profiling	T-cell/B-cell receptor RNA	Immune response, antigen specificity



Single Cell ATAC-seq is used to perform chromatin profiling of tens of thousands of single cells in parallel to allow researchers to see how chromatin compaction and DNA-binding proteins regulate gene expression at high resolution.

Next Generation Sequencing Instrumentation

The Genome Analysis and Technology Core NGS services located in UVA's Pinn Hall 1044 currently includes three benchtop Illumina sequencers. The Miseq, NextSeq500 and a new NextSeq2000 (NS2K). All runs currently performed on the NextSeq500 will be moved to the new NS2K platform after validation phase.

Platform specs and run comparison:

Table 1: Pricing of a Single cell project on different flow cells NextSeq 500 Vs. NextSeq2000

Specs	NextSeq 500 Flow cell -- 150Cycle kit	NextSeq 2000 P2 Flow cell -- 100Cycle kit	NextSeq 2000 P3 Flow cell -- 100Cycle kit
Number of Samples	4	4	4
Cells/Sample	5,000	5,000	5,000
Reads/Cell	50,000	50,000	50,000
Reads/Sample	250,000,000	250,000,000	250,000,000
Project Size (reads)	1,000,000,000	1,000,000,000	1,000,000,000
Expected output Reads/run)	400,000,000	400,000,000	1,000,000,000
Number of runs	3	3	1
Time (hrs) to Sequence per run	18	13	19
Kit cost (list price)	$3,100	$1,420	$3,250
Cost/Sample	$2,325	$1,065	$813
Total reagent kit Cost of project	$9,300	$4,260	$3,250

Table 2: Number of samples

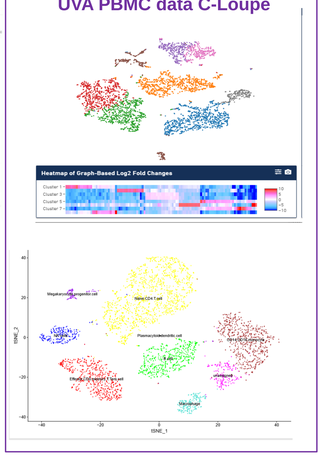
Application	NS500	NS2K
Single Cell Gene Expression 4,000 cells, 25,000 reads/cell	4 samples	10-12 samples
Whole Exome Sequencing 50X mean target coverage	16 samples	44 samples
Shotgum Metagenomics 50M reads/sample	8 samples	22 samples

Single Cell Data analysis: 10X Genomics provide free access to the Cell Ranger and C-loupe that are quick tools (30 min using Rivanna computing support nodes) to look at the quality of your data set. Additional Analysis is required.

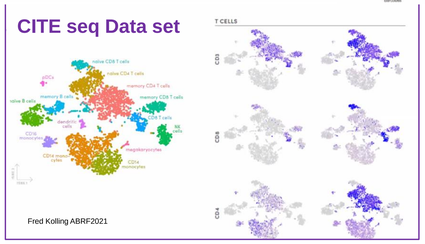
Cell Ranger pipeline- Web summary

- Do the number of cells with data match your expectations?
- Have you sequenced enough?
  - Rarefaction/sequencing saturation curves
- Method-specific metrics
  - 10x: reads in cell-containing droplets vs background

UVA PBMC data C-Loupe



CITE seq Data set



Sc.ATAC-seq adds regulatory information to the single cell data seq: